

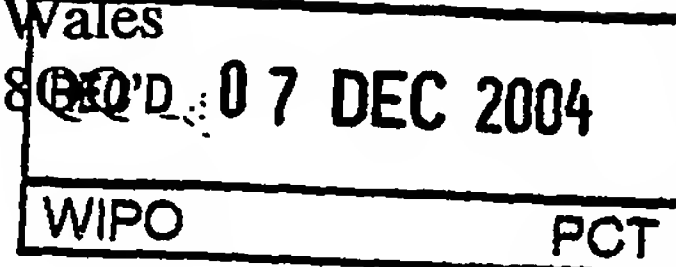


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INVESTOR IN PEOPLE

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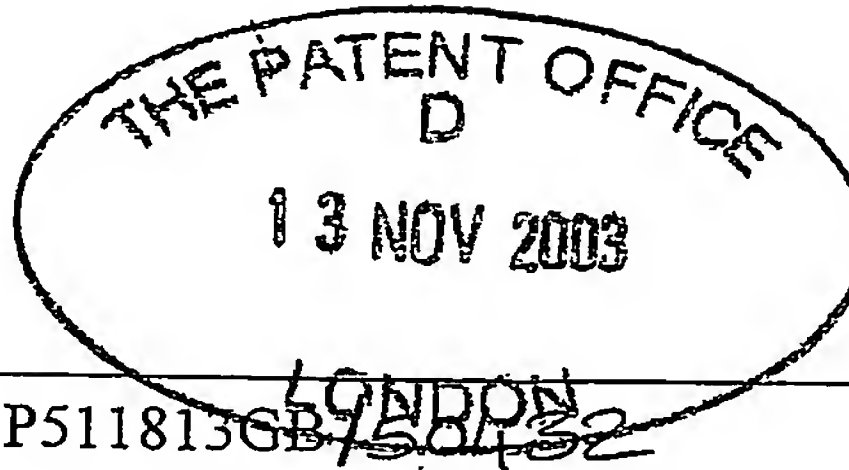
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Cardiff Road
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1. Your reference

P511813GB/50432

2. Patent application number

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0326520.4

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Compton Developments Ltd
P.O Box 425
Swansea
SA6 8YH

Patents ADP number (if you know it)

08376527001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

Molluscicidal and Anti-Barnacle Compounds

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

WITHERS & ROGERS
Goldings House
2 Hays Lane
London
SE1 2HW

Patents ADP number (if you know it)

1776001 ✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- See note (d))

YES

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Continuation sheets of this form

Description 32

Claim(s) 11

Abstract

Drawing (s) 3

23

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77) 1

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature *Withers & Rogers* Date *13/11/03*

12. Name and daytime telephone number of person to contact in the United Kingdom

Adrian Tombling

020 7663 3500

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Molluscicidal and Anti-Barnacle Compounds

The present invention relates to the use of one or more compounds as a molluscicidal and/or mollusc-repellant agent. The present invention also relates to a molluscicidal and/or mollusc-repellant agent comprising one or more of the compounds of the present invention. The present invention also relates to the use of one or more compounds as an anti-barnacle agent. The present invention also relates to an anti-barnacle composition comprising the one or more compounds. The present invention also relates to the use of a plant extract as an anti-barnacle agent.

Molluscs, especially slugs and snails cause considerable damage to crops and plants, and are therefore a pest to domestic gardeners as well as farmers. Current methods of controlling slugs and snails rely on the broad application of synthetic chemicals such as metaldehyde and methiocarb. There are a number of problems with using such chemicals, including the relatively high cost of the chemicals, the toxicity risks of storing and using such chemicals, and environmental problems, such as biodegradability and the toxic effects of the compounds on non-target organisms.

There is therefore a need for a new method of preventing damage to crops and plants caused by molluscs.

A wide range of plants are known to be of use in controlling aquatic molluscs. These are reviewed and listed in "Plant Molluscicides", edited by Mott K.E. (1987) UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease. John Wiley and Sons Ltd. These plants are not used to control terrestrial molluscs since most of them are dispersed by water and exert a surfactant effect. The book emphasises plants such as Endod (*Phytolacca dodecandra*) which produces a saponin. Most of these appear to work as aquatic molluscicides affecting surface tension at the gills of aquatic snails and leading to toxic haemolytic effects. Evidence suggests that they act by affecting cell membrane integrity (Henderson T.O., Farnsworth N.R and Myers T.C. (1987) Biochemistry of recognised molluscicidal

compounds of plant origin, Chapter 4, In *Plant Molluscicides*, Ed. K.E. Mott. pp.109-130). Such plants or their extracts are not used to control terrestrial molluscs, which are usually controlled by neurotoxins (Henderson I. And Triebkorn R. (2002) Chemical control of terrestrial gastropods. *Chapter 12.* in, *Molluscs as Crop Pests*, Ed. G.M. Baker, CABI Publishing, pp.1-31).

In particular, three plants indigenous to Nigeria, i.e. *Detarium microcarpum*, *Ximenia americana* and *Polygonum limbatum*, are known to have molluscicidal activity against aquatic snails (see Kela *et al.*, *Revue Elev. Med. vet. Pays trop.*, 1989, 42(2), 189-192; Kela *et al.*, *Pesticide Outlook*, 1995, 6(1), 22-27; Arthur *et al.*, *Slug & Snail, Pests in Agriculture BCPC Symposium Proceedings*, 66, 389-396, 1996). JP-A-6216477 discloses that extracts of plants belonging to *Pittosporaceae*, *Polygonaceae*, *Oleaceae* or *Gramineaceae* can be used in a composition to prevent aquatic molluscs from adhering to ships.

WO 00/04781 and US-A-5,290,557 relate to saponin containing plant extracts obtained from *Yucca shidegra*, *Quillaja saponaria* and *Hedera helix*.

Co-pending International PCT Patent Application PCT/GB03/001936 describes the use of plant material derived from a plant in the plant family *Caesalpinaceae*, *Olacaceae*, *Polygonaceae* or *Bursecaceae* as a terrestrial molluscicidal and/or mollusc-repellent agent.

The plant materials used include plant extracts such as alcoholic plant extracts but the actual active compound or compounds that have the molluscicidal and/or mollusc-repellent activity are unknown. This means that the plant materials used will have other compounds present and that these other compounds may have negative or

plant material. The structure of the active compound or compounds may be altered to improve their function, for example, by modifying the solubility, modifying the molluscicidal and/or mollusc-repellent activity, modifying the stability, etc.

When plants are damaged or bacterially infected they produce secondary metabolites, such as terpenes, as a defence against pathogens and invading pests. These secondary metabolites also behave as a chemical defence, for plants, against being eaten by herbivores. Plants therefore contain an untapped reservoir of pesticides that may have potential for use in the agrochemical industry.

The present application is directed to a specific group of compounds which generally fall into the category of terpenes. A terpene is a natural compound made up of isoprene units (5 carbon units) joined together head to tail in a regular pattern. Monoterpenes are the simplest family of isoprenoids and contain two isoprene units (10 carbon units). Sesquiterpenes are more complicated terpenes which are linked by 3 isoprene units (15 carbon units).

In US Patent No. 5,196,200 it is indicated some related sesquiterpenes behave as insect repellents against house flies and mosquitos. In particular, a mixture of bisabolene isomers was found to be effective against *Musca domestica* L. (Diptera: Muscidae) and *Aedes aegypti*.

Powell and Bowen (Slug and Snail Pests in Agriculture, BCPC Symposium Proceedings, 66, 261-236, 1996) selected certain monoterpenes as candidates for testing against the field slug, *D. reticulatum*, and found thymol, menthol and α -terpineol to be effective mollusc repellents. They also showed menthol, menthone and carvone to be potent molluscicides.

Iglesias *et al.*, (Proceedings of OILB/IOBC Working Group on Integrated Control of Soil Pests, Subgroup on Integrated Control of Slugs and Snails meetings, Lyon, France 2001) also found carvone to be a highly effective molluscicide when tested against the eggs of *D. reticulatum*. Carvone applied at a rate of 4.6 kg/ha was shown to induce death in 2 days.

Dodds (Slug and Snail Pests in Agriculture, BCPC Symposium Proceedings, No.66, 335-340, 1996) confirmed the antifeedant properties of (+) fenchone, a bicyclic monoterpene, isolated from the plant curled chervil (*Anthriscus cerefolium*), using electrophysiological techniques.

The identification of new and preferably more effective molluscicidal and/or mollusc repellent agents is desirable so that an effective and environmentally friendly agent can be used to prevent damage to plants and crops.

The present invention also relates to anti-barnacle agents. Barnacles are the major source of marine fouling. They normally settle on any surface offered up in the natural marine environment and are a major cause of the fouling of boat and ship hulls (Callow and Callow, Biologist, 49, 1-5, 2002). The fouling of hulls by marine organisms especially barnacles slows down ships and boats. This has accumulating costs in terms of fuel use and time lost, eventually leading to the increased costs of time out in dry dock to clean hull surfaces. Good anti-fouling paints can greatly reduce these costs. There is thus a vast demand for new non-toxic anti-fouling agents not only for shipping, including ocean liners, but also for offshore constructions, oil rigs, pulp and paper mills, water treatment plants and fish farming nets.

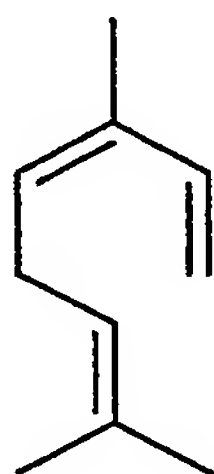
A very strong impetus for the invention of environmentally friendly anti-barnacle agents, is the fact that from 2003 the International Maritime Organisation has banned the use of the currently most widely used anti-barnacle paints containing tributyltin (TBT) on the basis of its environmental toxicity. All TBT based anti-barnacle paints are to be phased out by 2008. This is urgent since residues of TBT have been found throughout the world, particularly in sediments near shipping activity where it is especially toxic to shellfish.

According to a first aspect of the present invention there is provided a composition comprising an isolated compound and one or more carriers, for use as a molluscicidal and/or mollusc-repellent agent, wherein the compound is a terpene or oxygenated derivative thereof, the terpene is selected from one of the following general types:

- I) acyclic monoterpenes of the ocimene type;
- II) monocyclic sesquiterpenes of the bisabolene, germacrene or elemene type;
- III) acyclic sesquiterpenes of the farnesene type;
- IV) bicyclic sesquiterpenes of the santalene or carophyllene type; and
- V) furanosequiterpenes of the furanoelemene type, the furanoeudesma or furanogermacrene type.

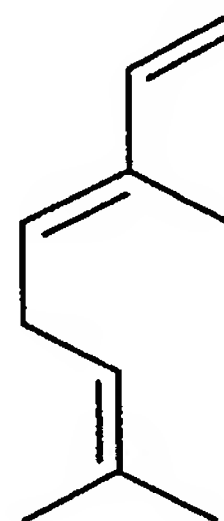
The compound used in the composition of the present invention must have molluscicidal and/or mollusc-repellent activity. It has been found that the specific terpene compounds identified above all have molluscicidal and/or mollusc-repellent activity.

Representative terpenes of the ocimene type include:



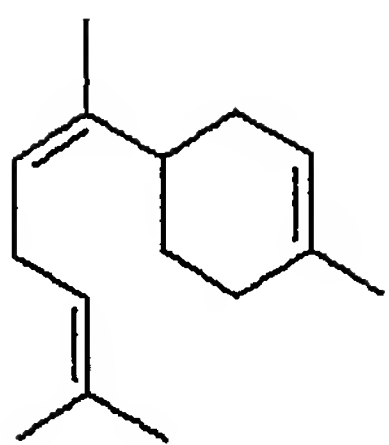
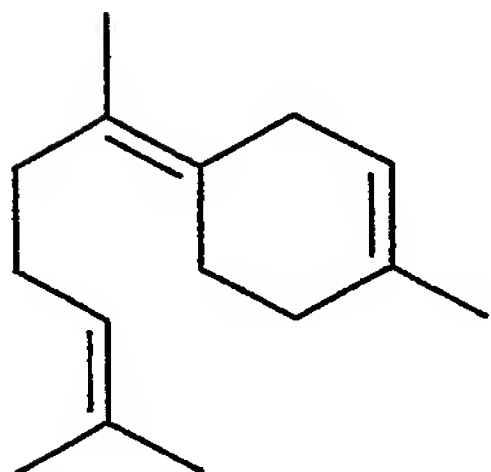
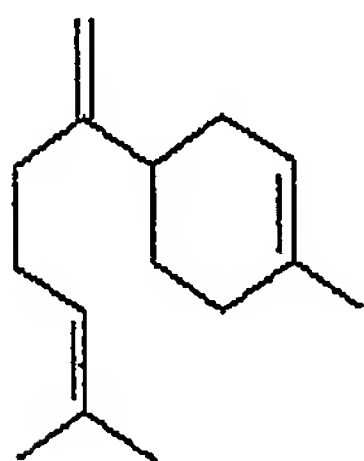
cis- β -Ocimene

and

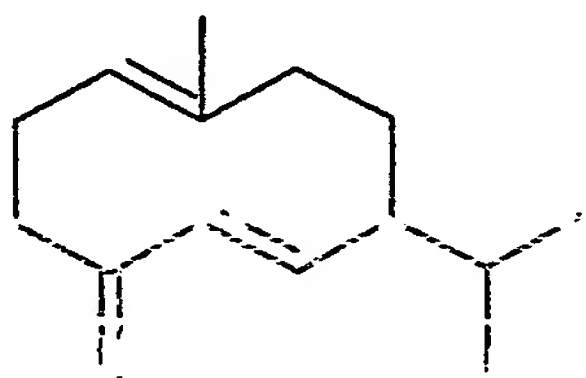


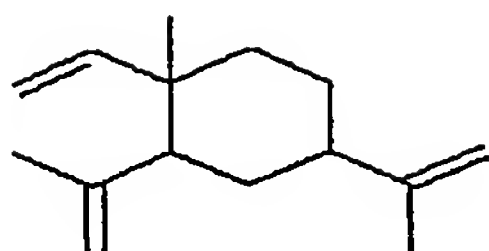
trans- β -Ocimene

Representative terpenes of the bisabolene type include:

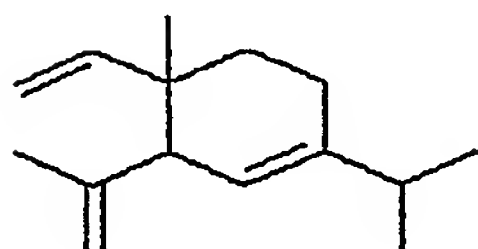
 α -bisabolene γ -bisabolene β -bisabolene

Representative terpenes of the germacrene type include:

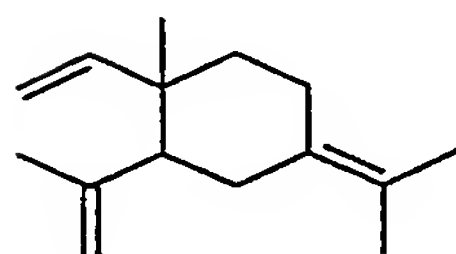




β -elemene

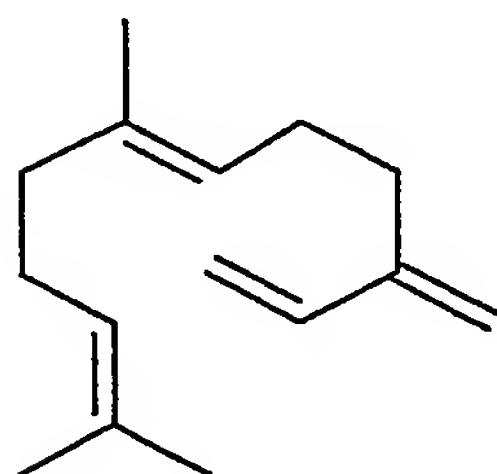


δ -elemene

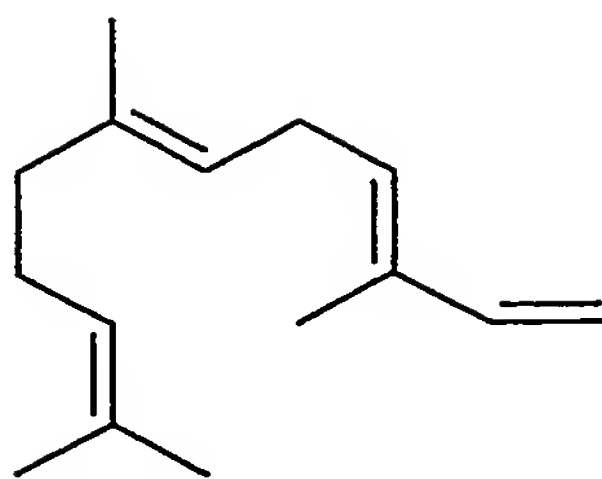


γ -elemene

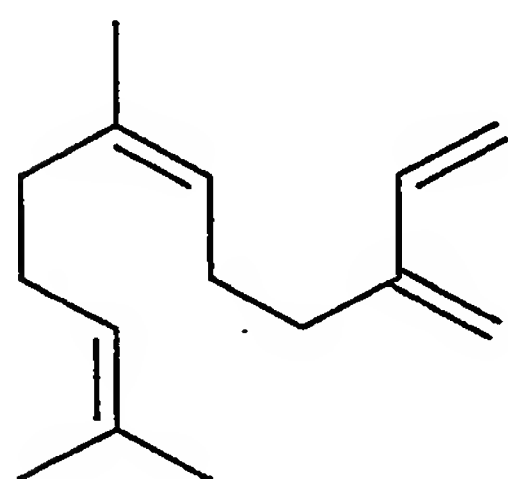
Representative terpenes of the farnesene type include:



α -farnesene
(trans, trans)

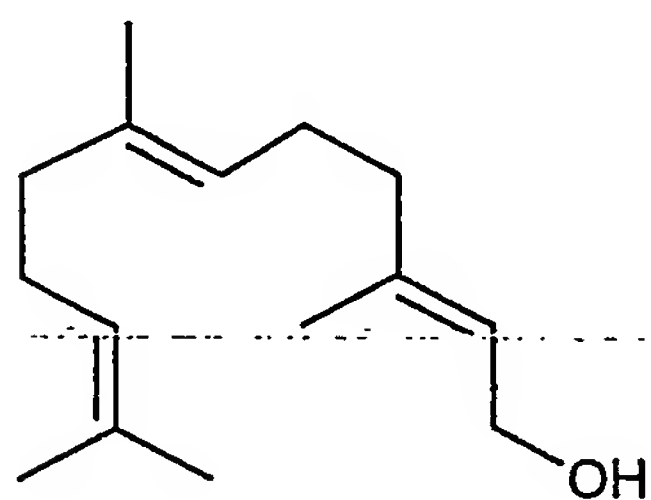


Trans- α -farnesene

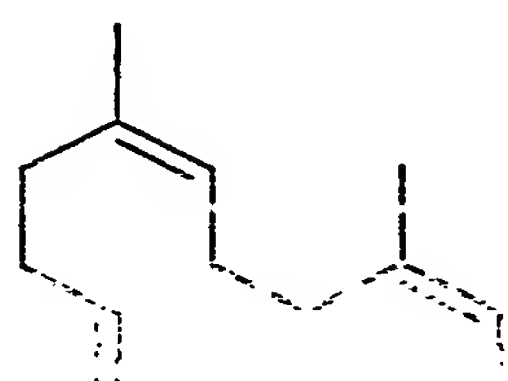


Cis- β -farnesene

and oxygenated derivatives thereof include:



farnesol (trans,trans) and

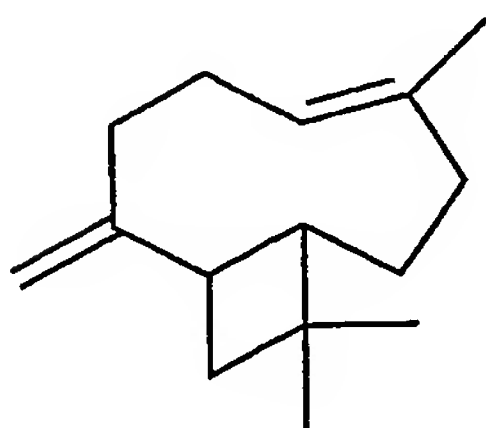


Representative terpenes of the santalene type include:



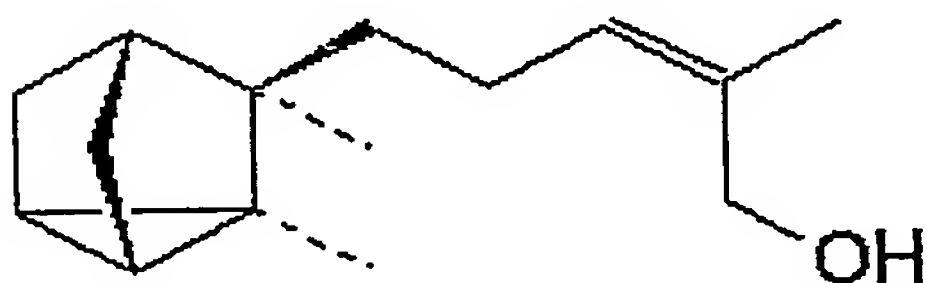
α -santalene

and of the carophyllene type include:



β -Carophyllene

and oxygenated derivatives of the santalene type include:



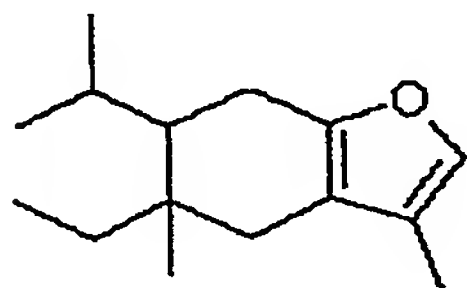
α -santalol

and

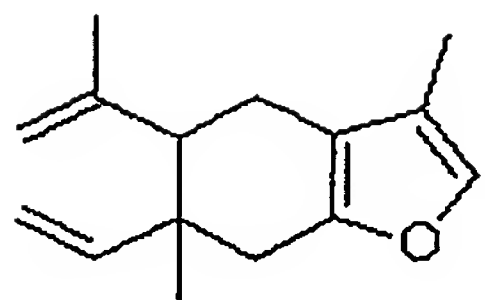


β -santalol

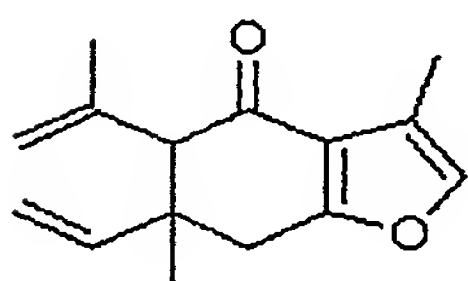
The general formulae of furanosequiterpenes of the furanoelemene type, furanoeudesma type and furanogermacrene type, respectively are indicated below along with a number of representative compounds thereof:



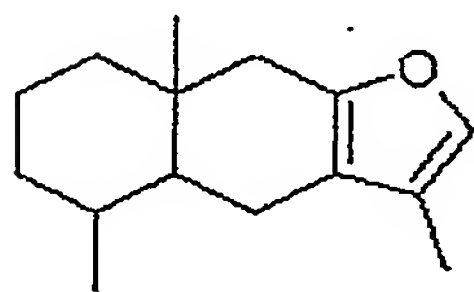
furanoelemene type, e.g.



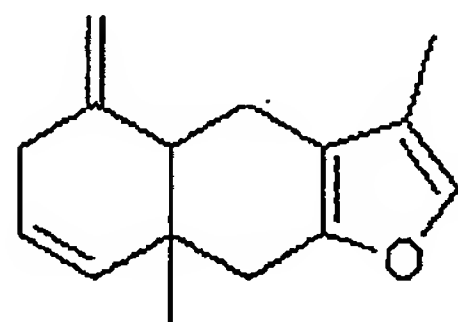
Curzerene, and



Curzerenone;

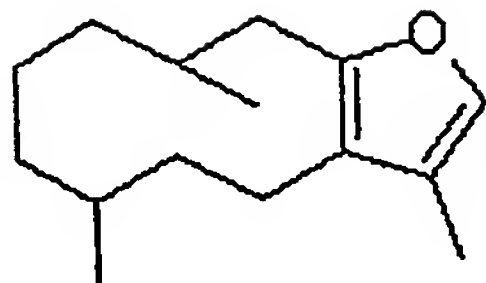


Furanoeudesma type, e.g.

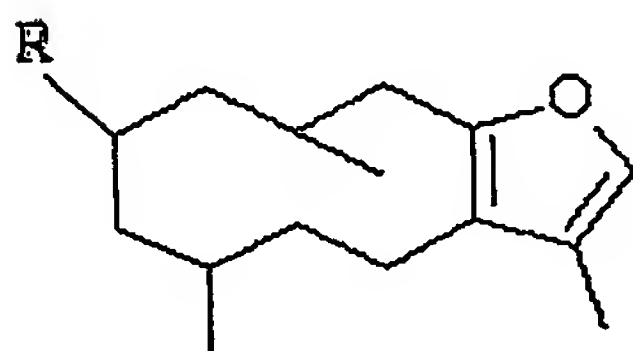


Lindestrene and





furanogermacrene type, e.g.



wherein R = H : Furanodiene
 R = OMe : 2 methoxy furanodiene
 R = O Ac : 2 acetoxy furanodiene.

It is particularly preferred that the compound used in the composition of the present invention is selected from the representative compounds indicated above. Modifications within the general formulae of the terpenes of the present invention can be made provided the modified compound has molluscicidal and/or mollusc-repellent activity. Those skilled in the art are well aware of appropriate modifications that can be made to the terpene compounds encompassed by the general formulae given above without destroying the activity of the compounds.

The term "isolated compound" means that the compound has been isolated from the other components with which it is naturally found. The one or more carriers are mixed with the compound to form the composition. In PCT/GB03/001936 plant material or crude plant extracts are used and the active molluscicidal compound has not been isolated.

Two or more of the isolated compounds may be used in conjunction as a molluscicidal and/or mollusc-repellent by using them simultaneously, sequentially or separately. The composition may comprise two or more of the terpene compounds.

The term "molluscicidal and/or mollusc-repellant" as used herein means that the agent kills and/or repels a terrestrial or aquatic mollusc. The term "terrestrial mollusc" as used herein means any mollusc that lives in a terrestrial environment for the majority of its lifetime. Particular examples of terrestrial molluscs include slugs of the genera *Arionidae*, *Milacidae*, *Boettgerillidae* and *Limacidae*, and snails of the genera *Helix*, *Cantareus*, *Bradybaena*, *Candidula*, *Carychium*, *Ceciloides*, *Cernuella*, *Cochlicopa*, *Cepea*, *Eobania*, *Discus*, *Euomphalia*, *Galba*, *Helicella*, *Helicigonia*, *Helicodiscus*, *Lacinaria*, *Monacha*, *Tymnaea*, *Retinella*, *Vertigo*, *Vitrea*, *Oxychilus*, *Physa*, *Succinea*, *Trichia*, *Vallonia*, and *Zonitoides*. A particularly preferred terrestrial mollusc is the grey field slug *Deroceras reticulatum*. The term "aquatic mollusc" as used herein means any mollusc that lives in an aquatic environment for the majority of its lifetime. The aquatic mollusc may be a fresh water or salt water mollusc. Particular examples of aquatic molluscs include molluscs of the genera *Biomphalaria*, e.g. *B. glabrata*, *B. pfeifferi*, *B. havanensis*, *B. sudanica*, *B. cenagophila*; *Bulinus*, *Ceirithium*, *Clarius*, *Dreissena*, e.g. *D. polymorpha*; *Heliosoma*, *Lymnea*, e.g. *L. stagnalis*, *L. natalensis*; *Marisa*, *Oncomelania*, e.g. *O. quadraxis*; *Phelidole*, *Pherna*, *Physa*, e.g. *P. occidentalis*; *Planorbis*, *Pomacea*, e.g. *P. Canaliculata*, *Tanebria*, and *Sepedon*.

The compound used in the composition of the present invention can preferably be obtained from a plant in the genera *Detarium*, *Ximenia*, *Polygonum*, *Commiphora* or *Boswellia*. In particular, it is preferred that the compound can be obtained from the plant *Detarium microcarpum*, *Ximenia americana*, *Polygonum limbatum*, *Commiphora* ~~malmei~~ *Commiphora* ~~malmei~~ or a *Boswellia* sp.

Commiphora molmol, *Commiphora guidotti* and *Boswellia sp.* are indigenous to the "Horn of Africa" (Somalia and Ethiopia). Furthermore, exudates from *Commiphora molmol* and *Commiphora guidotti*, when hardened, are known commercially as myrrh and scented myrrh. *Commiphora molmol*, *Commiphora guidotti* and *Boswellia sp.* are together herein referred to as odoriferous oleoresins and are known in Africa to repel insects and snakes. This has also been confirmed experimentally. *Commiphora molmol* has been shown to be larvicidal to mosquitoes (Massoud *et al.*, Journal of the Egyptian Society of Paracitology, 30, 101-115, 2000), while *Commiphora guidotti* has been found to have repellent and toxic effects against ticks (Maradufu, Phytochemistry, 21, 677-680, 1982; Carroll *et al.*, Entomol. Exp. Appl., 53, 111-116, 1989). It is indicated in PCT/GB03/001936 that the odoriferous oleoresins have molluscicidal and/or mollusc-repellent activity on terrestrial molluscs.

The compound used in the composition of the present invention can be obtained by isolating it from the appropriate plant indicated above. In particular, the majority of the compounds which can be used in the composition of the present invention can be isolated from *Commiphora molmol* or *Commiphora guidotti*. The compound can be isolated using any suitable preparative method. Methods of isolating such compounds are known to those skilled in the art and include chromatographic methods such as flash column chromatography and solid phase extraction columns. Nuclear Magnetic Resonance and Mass Spectrometry can be used to identify the individual compounds.

When the compound used in the composition of the present invention is isolated from a plant, substantially no contaminating plant material is present. Substantially no contaminating plant material means that less than 0.1% (w/w) of contaminating plant material is present.

The compound used in the composition of the present invention may be obtained from commercial sources such as Sigma-Aldrich Ltd (*cis*-Ocimene; *trans,trans*-Farnesol; and *cis,trans*-Farnesol) and RC Treat Ltd (*trans*- β -Ocimene; γ -bisabolene; Germacrene D;

Carophyllene; and α -Farnesene). The compound of the present invention may also be synthesised using standard chemical synthesis procedures.

The composition of the present invention comprises the compound and a carrier. The carrier is a heterologous carrier, i.e. the carrier is not associated with the compound in nature. In other words, the carrier is not associated with the compound in the plant from which the compound can be obtained. The type of carrier used will depend on how the composition is to be used, for example if the composition is to be used as a spray, it is preferred that the carrier is a suitable aqueous solution such as an alcoholic solution preferably comprising 1 to 10% alcohol in water. The carrier can also be an inert oil of plant origin, such as vegetable oil, corn oil and maize oil. Alternatively, the compound may be used in combination with a solid material.

In a preferred embodiment the carrier is a solid material. Any solid carrier material can be used such as a powder or a particulate.

It is particular preferred that the carrier is a particulate.

The term "particulate" as used herein refers to any substance which is in the form of particles which are of a sufficient size so as to act as an irritant to the movement of a terrestrial mollusc. In particular, it is preferred that the particulate is sand, sharp sand, pumice granules, sawdust, woodchips or corn cob chips. The particles, or at least the majority of the particles (for example about 90% of the particles by weight), are preferably between about 0.5 and 5 millimetres in diameter.

It is particularly preferred that the particulate is either sawdust or sharp sand.

the particulate forms a barrier to the molluscs. Therefore, by distributing the particles, which have been impregnated or coated with the compound, around plants to be protected, a barrier will be formed protecting the plants from molluscs.

The use of a particulate also has the added advantage of improving the friability, drainage and tilth of the soil. Furthermore, some particulates, e.g. sawdust and corncob, are biodegradable and degrade into a mulch which improves the soil.

In an alternative preferred embodiment the carrier is a liquid. When the composition is to be used in a liquid form, it is preferably sprayed. The advantage of spraying the composition is that it can be easily delivered over large areas. Furthermore, the composition can be sprayed onto soil, plants or seeds in order to kill and/or repel molluscs. In a preferred embodiment, the composition is in a formulation capable of being sprayed on to plants or seeds. A spray formulation may additionally comprise an emulsifying agent, such as tween 80.

The concentration of the composition is preferably selected so it is molluscidal/mollusc-repellant, but not toxic to non-mollusc species. More preferably the concentration is selected so that the composition is mollusc-repellant, but not molluscidal.

Preferably the composition contains between 0.1 and 5% compound, more preferably between 0.1 and 3% compound, most preferably between 0.25 and 1% compound.

The carrier is preferably selected for polarity. The inventors have surprisingly found that increasing the polarity of the carrier increases the molluscicidal and mollusc-repellant properties of the composition.

In a further embodiment of the present invention, the composition of the present invention is preferably combined with or used in conjunction with an effector agent.

The effector agent may be any agent which provides a beneficial effect to the plant or crop being protected from the molluscs. Suitable effector agents include fertilisers, fungicides and pesticides.

The composition of the present invention and effector agent may be used in conjunction by using them simultaneously, sequentially or separately. Preferably, the composition and the effector agent are combined together and used simultaneously.

The composition may be applied to surfaces such as wall, paths, etc. For example, the composition may be formulated as a paint-like preparations which can be painted or sprayed onto surfaces. The composition may be used in a aqueous environment by, for example, applying it to boat hulls, the sides of docks or fishing nets. Paint compositions comprising chemical molluscicidal and/or mollusc-repellent agents are known and one skilled in the art could modify these known paint compositions by incorporating the compound as defined above.

According to a second aspect of the present invention, there is provided the use of the isolated compound referred to in the first aspect of the present invention as a molluscicidal and/or mollusc-repellent agent.

Preferably the isolated compound is combined with one or more carriers as described with respect to the first aspect of the present invention.

As indicated above, the type of carrier used will depend on the agent, for example if the preparation is a spray, it is preferred that the carrier is a suitable aqueous solution such as an aqueous methanol or ethanol solution, or an inert oil of plant origin, such as vegetable oil, corn oil and maize oil.

In accordance with the first aspect of the present invention the isolated compound may be used in combination with an effector agent. The effector agent is as defined above with respect of the first aspect of the present invention.

In accordance with the first aspect of the present invention the isolated compound may be used as a molluscicidal and/or mollusc-repellant of both aquatic and terrestrial molluscs.

According to a third aspect of the present invention, there is provided the use of plant material derived from a plant in the plant family *Caesalpinaceae*, *Olaceae*, *Polygonaceae* or *Burseraceae* as an anti-barnacle agent.

It has been found that the use of plant material derived from plants within the above mentioned plant families acts as an anti-barnacle agent. It is assumed that the plants contain an anti-barnacle activity. The activity may be a single compound or a group of compounds.

The plant materials have previously been used as molluscicidal and/or mollusc-repellent agents but as the physiology of molluscs is substantially different from that of barnacles, it is surprising that the plant materials have anti-barnacle activity.

The term "anti-barnacle" as used herein means that the agent kills and/or repels barnacles. The term "barnacle" as used herein means any marine crustacean of the class *Cirripedia*, that can attach itself to a solid surface such as the hull of a boat, sea walls, etc. Particular examples of barnacles include *Balanus amphitrite*.

Preferably, the plant material is obtained from a plant in the genera *Detarium*, *Ximenia*, *Polygonum*, *Commiphora* or *Boswellia*. In particular, it is preferred that the plant material is obtained from the plant *Detarium microcarpum*, *Ximenia americana*, *Polygonum limbatum*, *Commiphora molmol*, *Commiphora guidotti* or a *Boswellia* sp.

Most preferably the plant materials is obtained from *Commiphora molmol* or *Commiphora guidotti*.

The plant material used in the present invention may comprise substantially the whole plant or particular parts of the plant, such as resinous exudates, that have anti-barnacle activity. Preferred parts include the bark, leaves or shoot of the plant. Preferably, such plant material is ground to particles or to a powder before use. The particles are preferably of a few millimetres in diameter (e.g. from 0.5 to 10 mm in diameter).

Alternatively, it is preferred that the plant material is an extract derived from the plant, wherein the extract has anti-barnacle activity. The extract is preferably an alcoholic extract and may be obtained using standard procedures for obtaining alcoholic extracts of the plant. In particular, methods for obtaining such an alcoholic extract are well known to those skilled in the art. Alternatively, it is preferred that the plant material is an alcoholic extract of an essential oil of the plant. Essential oils are the volatile, organic constituents of fragrant plant matter. Essential oils are generally extracted from plant by two main methods, distillation (steam, water or dry distillation) and cold pressing. A plant extract containing mainly oils can also be prepared using solvents, carbon dioxide extraction or hydrofluoroalkanes. Tincture plant extracts can be made by macerating the plant materials and extracting using aqueous, ethanolic solvents (70%-90% ethanol in water) and left for a period of time, after which the solid debris is filtered.

In a particular preferred embodiment, the plant material is a substantially isolated compound or mixtures of compounds having anti-barnacle activity. Methods of isolating such compounds are known to those skilled in the art and include chromatographic methods. For example, the essential oils of the plant can be extracted

Nuclear Magnetic Resonance and Mass Spectrometry can be used to identify the individual compounds.

The term "substantially isolated" means that the molluscicidal and/or mollusc-repellant compound or compounds are substantially isolated from the plant. Preferably, the molluscicidal and/or mollusc-repellant compound or compounds comprise less than 5% (w/w), more preferably less than 1% (w/w) of contaminating plant material.

The plant material of the present invention is preferably used in combination with a carrier. The type of carrier used will depend on how the plant material is to be used, for example if the plant material is to be used as a spray, it is preferred that the carrier is a suitable aqueous solution such as an alcoholic solution preferably comprising 1 to 10% alcohol in water. The carrier can also be an inert oil of plant origin, such as vegetable oil, corn oil and maize oil. Alternatively, if the plant material is to be used as a paint, any paint base can be used.

The plant material is preferably applied to surfaces to repel molluscs from the surfaces. The plant material may be made into suitable preparations for applying to surfaces such as paint-like preparations which can be painted or sprayed onto surfaces.

In a further embodiment of the present invention, the plant material is preferably used in conjunction with an effector agent.

The effector agent may be any agent which provides a beneficial effect. Suitable effector agents include complementary anti-barnacle agents.

Preferably, the plant material and the effector agent are combined together and used simultaneously.

It is particularly preferred that the plant material is used as a paint-like preparation which can be painted or sprayed onto surfaces. It is also preferred that the preparation is used in a aqueous environment by, for example, applying it to boat hulls, the sides of

docks or fishing nets. Paint compositions comprising chemical anti-barnacle agents are known and one skilled in the art could modify these known paint compositions by incorporating the plant material as defined above.

According to a fourth aspect of the present invention there is provided an anti-barnacle composition comprising an isolated compound in combination with one or more carriers for use as an anti-barnacle agent, wherein the compound is a terpene or oxygenated derivative thereof, as defined above with respect to the first aspect of the present invention.

The composition is preferably suitable for applying to surfaces that come into contact with barnacles.

The type of carrier used will depend on how the compound is to be used, for example if the anti-barnacle composition is to be used as a spray, it is preferred that the carrier is a suitable aqueous solution such as an alcoholic solution preferably comprising 70 to 90% alcohol in water. Alternatively, if the anti-barnacle composition is to be used as a paint, any paint base can be used.

The anti-barnacle composition is preferably applied to surfaces to repel barnacles from the surfaces. The plant material may be made into suitable preparations for applying to surfaces such as paint-like preparations which can be painted or sprayed onto surfaces.

The anti-barnacle composition preferably comprises between 0.1 and 50% v/v compound, more preferably between 3 and 25 %, most preferably between 6 and 25%.

In a further embodiment of the present invention, the anti-barnacle composition is

Preferably, the anti-barnacle composition and the effector agent are combined together and used simultaneously.

It is particularly preferred that the anti-barnacle composition is used as a paint-like preparation which can be painted or sprayed onto surfaces. It is also preferred that the preparation is used in a aqueous environment by, for example, applying it to boat hulls the sides of docks or fishing nets. Paint compositions comprising chemical anti-barnacle agents are known and one skilled in the art could modify these known paint compositions by incorporating the anti-barnacle composition as defined above. As will be appreciated by those skilled in the art, as the anti-barnacle composition is used as an anti-barnacle agent, it preferably comprises a carrier which enables it to be applied to surfaces on which the attachment of barnacles is to be reduced or prevented. Carriers such as paint bases are particularly suitable.

According to a fifth aspect of the present invention, there is provided the use of the isolated compound referred to in the fourth aspect of the present invention as an anti-barnacle agent.

Preferably the isolated compound is combined with one or more carriers as described with respect to the fourth aspect of the present invention. The isolated compound is preferably used in the concentrations described with respect to the fourth aspect of the present invention.

In accordance with the fourth aspect of the present invention the isolated compound may be used in combination with an effector agent. The effector agent is as defined above with respect of the fourth aspect of the present invention.

The present invention is now described, by way of example only, with reference to the accompanying figures in which:

Figure 1 shows the slug induced lettuce leaf damage before and after spraying with a 3% aqueous solution of trans- β -ocimene.

Figure 2 shows the barnacle settlement results when using an extract from *Commiphora gudiotti* (extract H).

Figure 3 shows the barnacle settlement results when using an extract from *Commiphora molmol* (extract M).

Figure 4 shows the barnacle settlement results when using trans- β -ocimene (compound O).

Figure 5 shows the use of ethanol and seawater in the barnacle settlement experiments by way of a control.

EXAMPLES

EXAMPLES - Molluscicidal and mollusc-repellent compounds

MATERIALS AND METHODS

Reagents

Absolute ethanol and hexane (HPLC grade) were obtained from Fischer Scientific (UK).

The following reagents and chemicals were purchased from Sigma-Aldrich limited, Dorset: Dimethyl sulfoxide (D.M.S.O) (99.5% purity), Tween 80 (Polyoxyethlenesorbitan monooleate), *cis*- Ocimene (purity 70%), trans,trans-Farnesol (purity 96%), *cis*,trans-Farnesol (purity 95%), myrrh essential oil and opoponax

Pure Sandalwood essential oil (*santalum album*), Tisserand Aromatherapy, was purchased from Neal Yards Remedies, Cardiff.

Test animals

Adult *D. reticulatum* were collected from nearby fields and maintained in plastic trays lined with moist, unbleached, absorbent paper. They were housed in the dark and at a constant temperature of $10^{\circ} \pm 1^{\circ}\text{C}$. Slugs were regularly fed on a mixture of iceberg lettuce and carrots. Slugs, with a weight 300-600mg, were pre-starved for 24 hours and maintained constant temperature of $15^{\circ} \pm 1^{\circ}\text{C}$ prior to testing.

Preparation of test materials

The terpene oils listed, above, were prepared in different media as shown below:

i) Ethanol

A known weight of terpene oil was weighed into a 10 ml volumetric flask and diluted to volume with absolute ethanol.

ii) DMSO extracts (10%)

A known weight of terpene oil was weighed into a 10 ml volumetric flask containing 1g of dmso. This was lightly mixed and diluted to volume with water resulting in essential oil final concentrations of 0, 0.5, 1 and 5 % w/v. This was vortex mixed to form an emulsion.

iii) Tween 80 extracts (0.2%)

A known weight of terpene oil was weighed into a 10 ml volumetric flask containing 0.02g of tween 80. This was lightly mixed and diluted to volume with water. This was vortex mixed to form an emulsion.

iv) Tween 80 extracts (0.5%)

A known weight of terpene oil was weighed into a 10 ml volumetric flask containing 0.05g of tween 80. This was lightly mixed and diluted to volume with water. This was vortex mixed to form an emulsion.

v) **Water extracts**

A known weight of terpene oil was weighed into a 10 ml volumetric flask and diluted to volume with water. This was vortex mixed to form an emulsion.

BIOASSAY

The technique described here is a no choice-feeding bioassay using circular discs prepared from lettuce leaves. Selected terpenes were tested against *D. reticulatum* slugs and evaluated for their repellent/antifeedant and molluscicidal properties.

Leaf Disc Assay Method

Lettuce leaf discs (1.4cm²) were placed in petri dishes lined with water saturated filter paper (9cm² diameter). A known concentration of terpene oil, solubilised in the appropriate media, was prepared and a fixed volume (50µl) added by pipette, onto individual lettuce leaf discs. Any residual solvent was left to evaporate for a minimum time of 30 minutes.

Slugs, previously starved for 24 hours, were introduced to the petri dishes and placed in an environmentally controlled chamber (15°C: 12 hours day, 15°C: 12 hours night) for 24 hours.

The amount of leaf disc damaged (eaten) was quantified by comparing digital photographs of treated leaf discs with untreated (control) leaf discs, using photographic computer software. The experiment was replicated 10 times for each terpene.

The antifeedant effect of various media was determined by comparison to untreated leaf discs. A positive R I value indicates a reduction in feeding (antifeedant/ repellent) whereas a negative value indicates an increase in feeding behaviour (phagostimulant). Corrections for mortality were done the Abbotts formula (Abbott, 1925).

$$\text{Corrected Mortality (\%)} = \frac{\text{observed \% mortality} - \text{control \% mortality}}{100 - \text{control \% mortality}} \times 100$$

BIOASSAY ASSAY RESULTS: Leaf Disc Assay

Example 1

Table 1. Change in feeding behaviour and molluscicidal activity after treating leaf discs treated with different media

Solvent	Repellency Index (%)	Corrected Mortality (%)
Ethanol	0.4	0
Aqueous dmso (10%)	- 2	0
Aqueous dmso (2.5%)	5	0
Aqueous tween 80 (0.2%)	0.5	0
Aqueous tween 80 (0.5%)	8	0
Water	0	0

The different media, used to solubilise the terpenes, had no adverse repellent or antifeedant effects towards the feeding behaviour of *D. reticulatum*.

Example 2.

Table 2. Change in Feeding behaviour and mortality after treating leaf discs with the *trans* isomer of β -Ocimene (0.45%) in different media

Sample description	Solubilising Media	Repellency Index (%)	Corrected Mortality (%)
<i>trans</i> - β -Ocimene (0.45%)	Ethanol	35	0
	Aq. Tween 80 (0.5%)	73	10
	Aq. Tween 80 (0.2%)	91	20
	Aq. Dmso (2.5%)	91	20
	Aq. Dmso (10%)	90	70
	Water	100	90

Example 2 confirms the repellent and molluscicidal nature of the monoterpene *trans*- β -ocimene. When formulated as an aqueous emulsion, with a nonionic surfactant (0.2 and 0.5% tween 80), it reducing leaf consumption by 73 to 91 %. Changing the media to aqueous dmso (10%), increased the molluscicidal behaviour, whilst maintaining its repellency properties. The molluscicidal properties of ocimene increased, again, when only water was used as an emulsion formulation. No leaf disc consumption was observed resulting in 100% repellency. There is clear relationship between the repellency properties of *trans*- β -ocimene and the polarity of the media that is used to formulate the treatments. It is therefore concluded that increasing the polarity of the media increases the repellency and molluscicidal properties of *trans*- β -ocimene.

Table 3. Change in Feeding behaviour and mortality after treating leaf discs with the *trans* isomer of β -Ocimene (0 to 3%) in aqueous dmso (10%)

Media	<i>trans</i> - β -Ocimene (%)	Repellency Index (%)	Corrected Mortality (%)
Aq. Dmso (10%)	0 %	3	0
	0.25	1	0
	0.5	1	0

Example 3 shows the effect of ocimene concentration on the antifeedant/molluscicidal behaviour against *D. reticulatum*. In general there is an increase in mortality and a decrease in leaf disc consumption on increasing the ocimene concentration.

Example 4.

Table 4. Change in Feeding behaviour and mortality after treating leaf discs with the *cis* isomer of β -Ocimene

Sample description	Solvent	Repellency Index (%)	Corrected Mortality (%)
<i>cis</i> - β -Ocimene (0.35%)	Aq. Tween 80 (0.2%)	84	15

Example 2 and 4 both indicate a strong repellent/antifeedant effect for the stereoisomers of β -ocimene (*cis* and *trans*). Comparing their solubilisation in aqueous tween 80 (0.5%) showed that the amount of lettuce leaf consumed was reduced by 73% for *trans*- β -ocimene and by 84% for the corresponding *cis* isomer. Low mortalities were obtained for both the *trans* and *cis* ocimene stereoisomers.

Example 5.

Table 5. Change in feeding behaviour after treating leaf discs with selected mono and sesquiterpenes, solubilised in ethanol

Sample description	Repellency Index (%)	Corrected Mortality (%)
<i>trans</i> - β - Ocimene (0.1%)	5	0
<i>trans</i> - β - Ocimene (0.45%)	35	0

<i>trans</i> - β -Ocimene (0.9%)	95	5
α - farnesene (0.7%)	60	10
α - farnesene (2.7%)	97	50
<i>trans, trans</i> farnesol (0.96)	96	0
<i>cis, trans</i> farnesol (0.95%)	77	0
Germacrene D (0.4%)	77	0
γ - bisabolene (0.7%)	70	0
Carophyllene (0.96%)	40	15

All of the terpenes, solubilised in ethanol, gave a good measure of repellency against the feeding behaviour of *D. reticulatum*, with the exception of the lower concentrations of ocimene (0.1 and 0.45%) and carophyllene yielding 5, 35 and 40% reduction in the consumption of lettuce leaf discs, respectively.

All the other terpenes tested gave over 70% repellency against the feeding molluscs. High slug mortalities (50%), using ethanol solvent, were observed when high levels of farnesene (2.7%) was applied to the lettuce leaf discs.

Example 6.

Commiphora guidotti (opoponax) essential oil contains high levels of the sesquiterpene α -santalene (22. to 26%). The alcoholic analogues (α and β santalols) occur at high levels in sandalwood essential oil. These alcohols, together, accounted for 90% of the essential oil chemical composition.

Sample description	Solvent	Repellency Index (%)	Corrected Mortality (%)
Sandalwood Oil (0.5%)	Aq. dmso (10%)	91	0

Example 6 signifies the potent repellent/antifeedant nature of sandalwood essential oil, when solubilised in aqueous dmso (10%). This change in feeding behaviour is considered to be due to the presence of alcoholic sesquiterpenes, α and β santalol, which together account for 90% of the total compounds present in sandalwood essential oil.

Spray Trials

Method

To evaluate the efficacy of *trans*- β -ocimene emulsions using peat substrates lettuce leaves were sprayed with 12 ml of an aqueous ocimene emulsion (3%). Once sprayed the leaves were placed in plastic trays containing peat soil and left for 30 minutes to remove the excess liquid drops. After this time period two adult slugs, previously starved for 24 hours, were added to each plastic tray and left for 24 hours.

A control (water) was treated similarly. Four replicates were prepared for each treatment.

Repellency indices were calculated as described for the leaf disc bioassay.

RESULTS

Example 7.

Table 7. Evaluation of aqueous emulsions of trans- β -Ocimene as a repellent spray against *D. Reticulatum*.

Treatment	Replicate No	Leaf damage (%)	Mean Leaf damage (%)	Repellency Indices (%)
<i>trans</i> - β -Ocimene in water (3%)	1	0.99	7.25	76.5
	2	18.69		
	3	9.33		
	4	0.00		
Control	1	57.64	30.86	
	2	33.28		
	3	15.75		
	4	16.77		

Figure 1 shows a photographs of slug induced lettuce leaf damage, after spraying with 3% aqueous trans- β -ocimene. Examples 6 and 7 show the repellency effects observed when spraying lettuce with an aqueous emulsion of 3% trans- β -ocimene.

Significant protection, against the feeding activity of *D. reticulatum* molluscs, was observed over a 24 hour period (77%).

No mortality was observed in any of the peat saturated containers.

EXAMPLES - Anti-barnacle agent

Investigations show that extract of Myrrh resin from *Commiphora molmol* and from scented Myrrh *Commiphora gudiotti* or "Haddi", are potent inhibitors of the settlement of the larvae of the barnacle *Corbicula* and therefore a potential anti-barnacle agent.

The settlement of the barnacle *Balanus Amphitrite* was tested in multi-welled plates in the laboratory. Myrrh and scented Myrrh were extracted in ethanol and 2ml aliquots from a range of concentrations (50%v/v to 1.5%v/v) were evaporated to dryness in each well of a multi wellled plate. Ethanolic trans- β -Ocimene a component of scented Myrrh was also applied in the manner over a range of concentrations (50%v/v to 1.5-%v/v). After evaporation saltwater containing active cyprid larvae was introduced to each well and their settlement and activity measured after 24hrs.

Figure 2 shows the results of a settlement experiment using an extract from Haddi (*Commiphora gudiotti*). After treatment with extract H from Haddi, the majority of larvae were classified as non-active after the 24hr incubation. This means they are either dead or not moving with only slight internal movement. As the dose decreases, the level of non-active larvae decreases, while the level of active swimmer increases. This indicates a dose-dependent effect. There is no settlement except for 1 individual at the top concentration but this is probably a rogue. There was an oily meniscus on the surface of the wells, presumably from the extract. Overall, this extract at the concentrations tested is toxic to barnacle larvae (*B. Amphitrite*).

Figure 3 shows the results of a settlement experiment using an extract from Myrrh (*Commiphora molmol*). Doses 50-12.5% v/v of extract M from Myrrh resulted in all the larvae becoming inactive. They were either dead or showing slight internal movement, but no limb movement. Doses 6.25 and 3.125% saw an increase in active larvae, with 3.125% having 84% active larvae and 16% settled. This indicates a dose-dependent response from the extract in terms of toxicity.

Figure 4 shows the results of a settlement experiment performed using compound trans- β -Ocimene (from *Commiphora gudiotti*). Compound O (trans- β -Ocimene) had some unusual effects on the larvae. This was possibly caused by an osmotic change as the body had swollen and increased in size and was too large for the cuticle, resulting in a strange 'mutation' effect. At the top two doses the larvae were all floating on the surface but were 'mutated'. As the dose decreased, the % non-active (including mutations) decreased and the amount of active and settled larvae increased, again indicating a dose-dependent response.

Both the ethanol and seawater controls (Figure 5) showed the majority of larvae active, with a small amount of settlement and virtually no dead larvae indicating a healthy batch of larvae was used in the experiment.

Conclusions

All the extracts tested had an inhibitory dose-dependent effect on larval settlement. Whether this is caused by toxic effects and is lethal or is inhibitory and reversible when removed from the solution is unclear. Further experiments at lower concentrations need to be carried out. Extract H appeared to have the best effect, as there was no settlement even at the lowest dose. Further individual compounds from this extract are currently being tested.

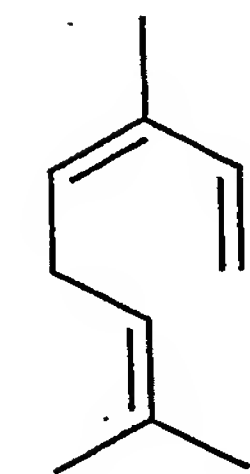
All documents cited above are incorporated herein by reference.

Claims

1. A composition comprising an isolated compound in combination with one or more carriers for use as a molluscicidal and/or mollusc-repellant agent, wherein the compound is a terpene or oxygenated derivative thereof, the terpene is selected from one of the following general types:

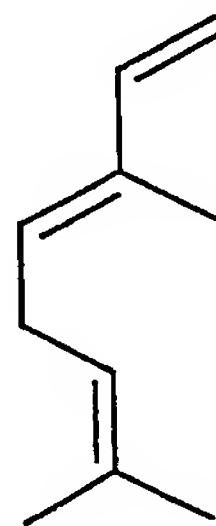
- I) acyclic monoterpenes of the ocimene type;
- II) monocyclic sesquiterpenes of the bisabolene, germacrene or elemene type;
- III) acyclic sesquiterpenes of the farnesene type;
- IV) bicyclic sesquiterpenes of the santalene or carophyllene type; and
- V) furanosequiterpenes of the furanoelemene type, the furanoeudesma or furanogermacrene type.

2. The composition of claim 1, wherein the compound is



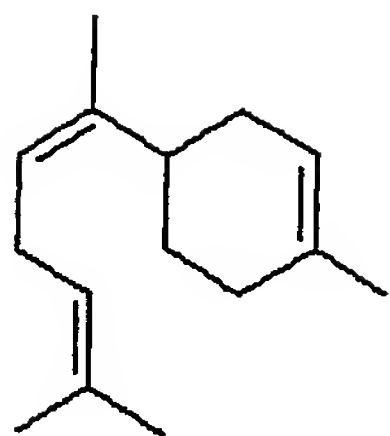
cis- β -Ocimene

or

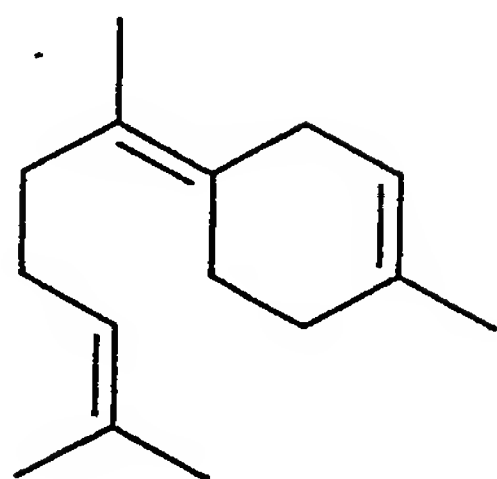


trans- β -Ocimene.

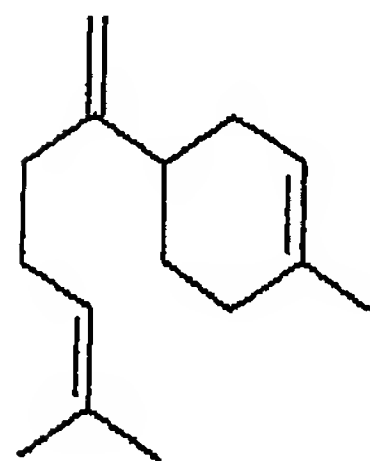
3. The composition of claim 1, wherein the compound is



α -bisabolene,

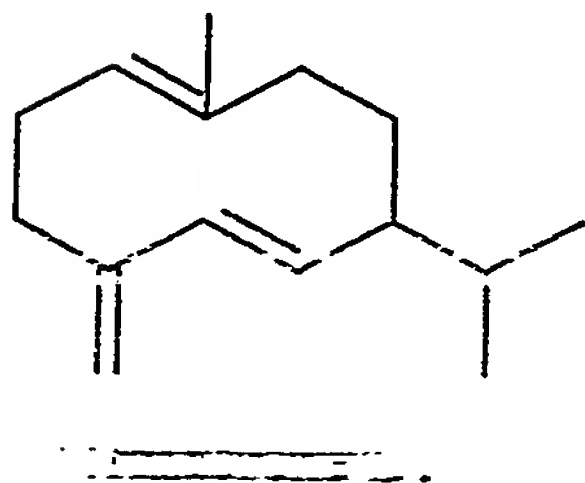


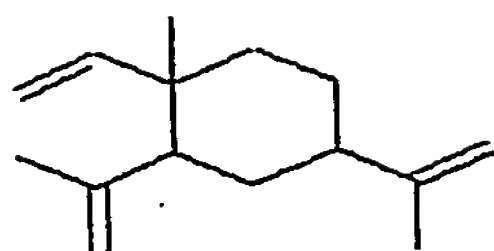
γ -bisabolene, or



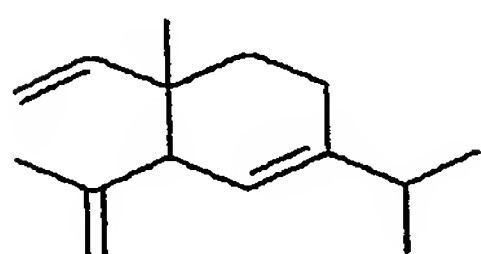
β -bisabolene.

4. The composition of claim 1, wherein the compound is

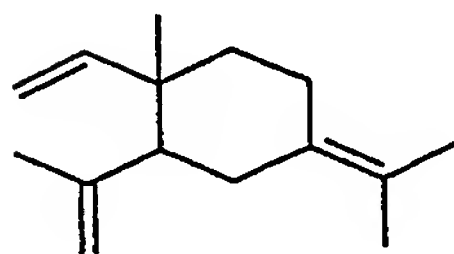




β -elemene,

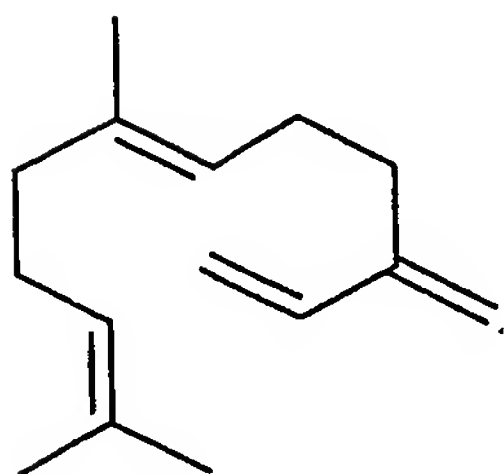


δ -elemene, or

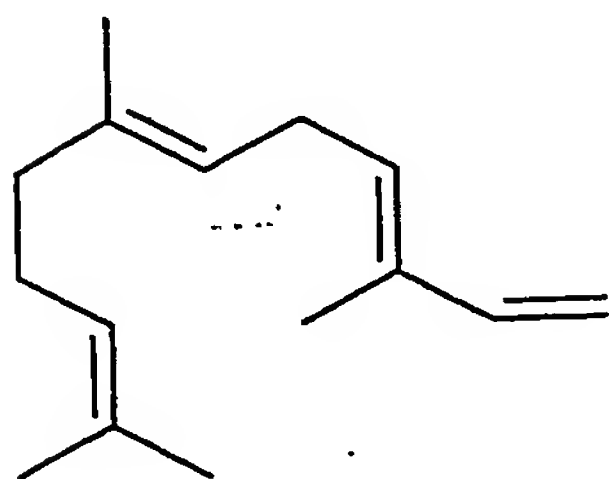


γ -elemene.

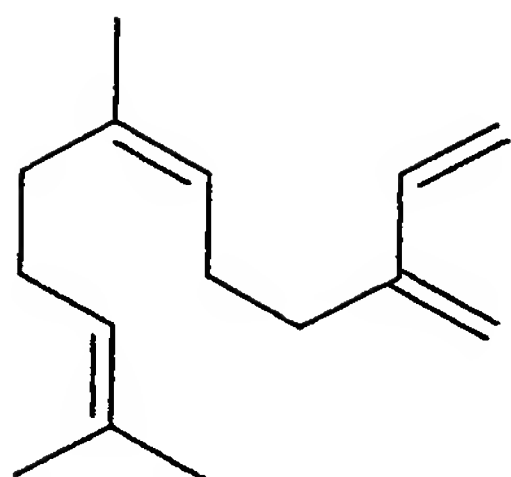
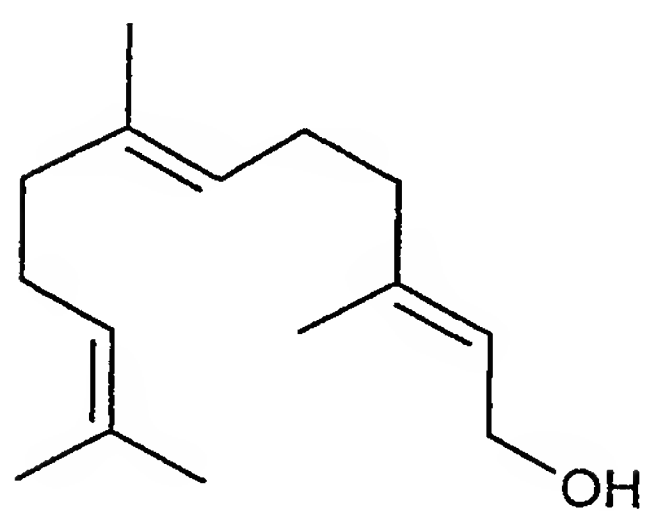
6. The composition of claim 1, wherein the compound is



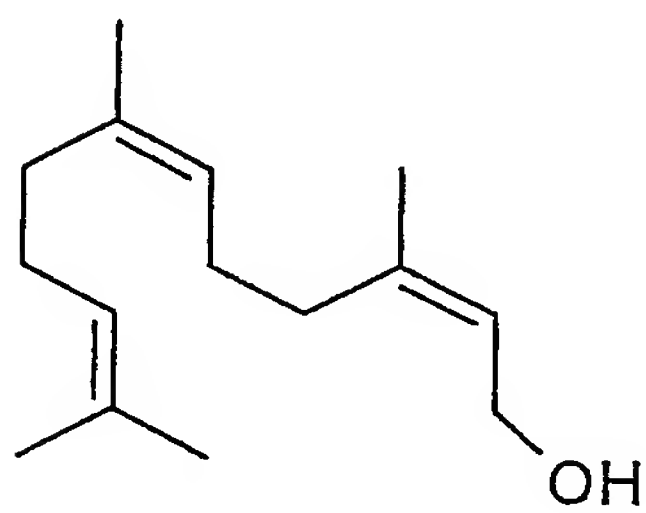
α -farnesene
(trans, trans),



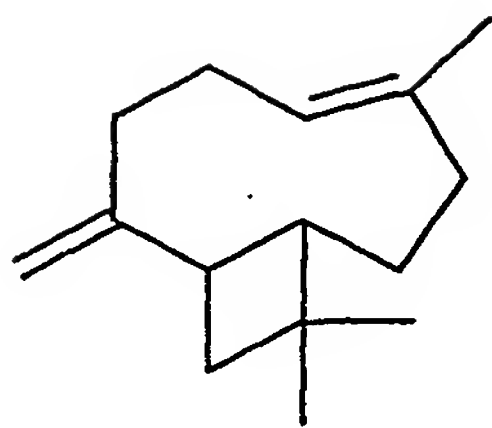
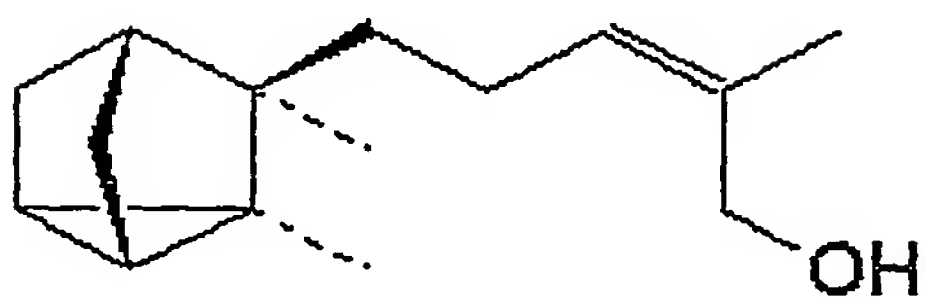
Trans- α -farnesene,

Cis- β -farnesene,

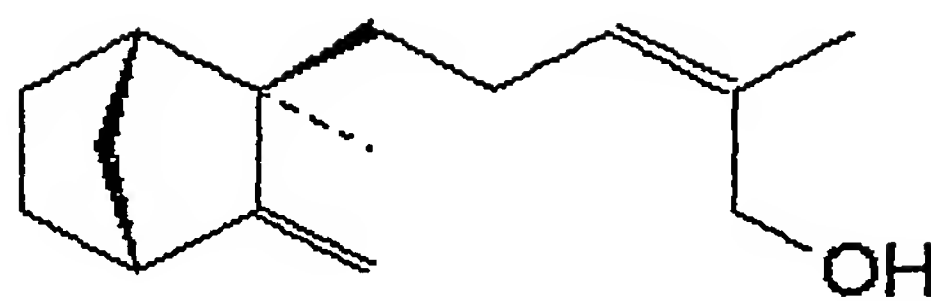
farnesol (trans,trans), or



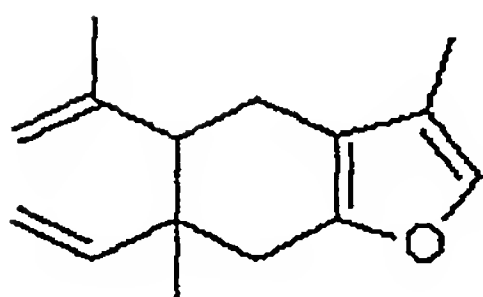
farnesol (cis,trans).

 β -Carophyllene α -santalol

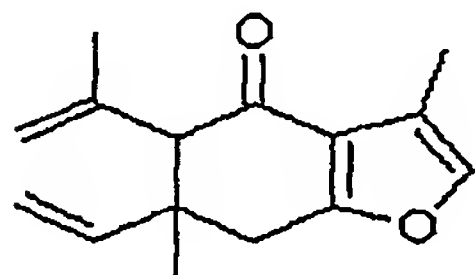
or

 β -santalol

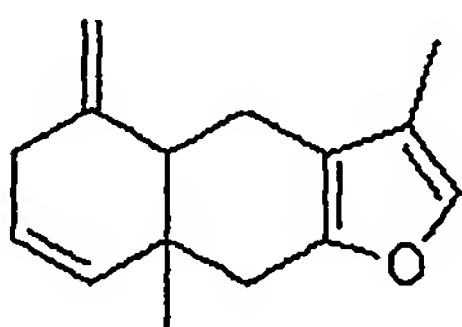
8. The composition of claim 1, wherein the compound is



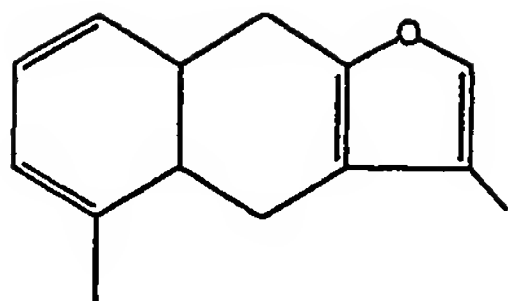
Curzerene,



Curzerenone,

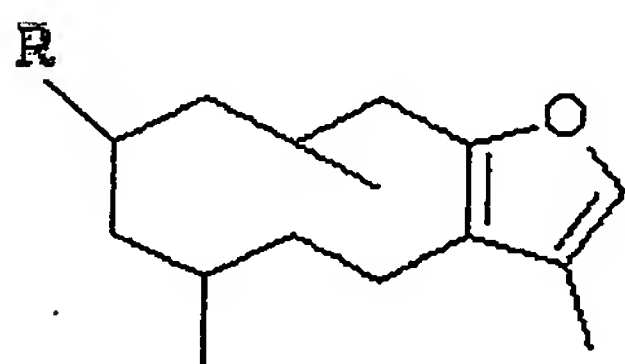


Lindestrene, or



Furanoeudesma-1,3-diene.

9. The composition of claim 1, wherein the compound is



wherein when R = H : Furanodiene
 R = OMe : 2 methoxy furanodiene
 R = O Ac : 2 acetoxy furanodiene.

10. The composition according to any one of the preceding claims, wherein the carrier is a heterologous carrier.

11. The composition according to any one of the preceding claims, wherein the compound is obtained from a plant in the genera *Detarium*, *Ximenia*, *Polygonum*, *Commiphora* or *Boswellia*.

12. The composition according to claim 11, wherein the plant is *Detarium microcarpum*, *Ximenia americana*, *Polygonum limbatum*, *Commiphora molmol*, *Commiphora guidotti* or a *Boswellia* sp.

13. The composition according to claim 12, wherein the plant is *Detarium microcarpum*.

14. The composition according to claim 12, wherein the plant is *Ximenia americana*.

15. The composition according to claim 14, wherein the particulate is sand, sharp sand, pumice granules, sawdust, wood chips or corncob chips.
16. The composition according to claim 14, wherein the particulate is sawdust.
17. The composition according to any one of claims 1 to 13, wherein the composition is in a formulation capable of being sprayed.
18. The composition according to claim 17, wherein the carrier is an aqueous solution comprising between 1 and 10% alcohol in water.
19. The composition according to one of the any preceding claims, in combination with an effector agent.
20. The composition according to claim 19 wherein the effector agent is a fertiliser or a pesticide.
21. The composition according to any of the preceding claims, which contains between 0.1 and 5%v/v compound.
22. The composition according claim 21, which contains between 0.1 and 3%v/v compound.
23. The composition according to claim 22, which contains between 0.25 and 1%v/v compound.
24. The use of the composition according to any one of the preceding claims as a molluscicidal and/or mollusc-repellent agent.
25. The use of an isolated compound as a molluscicidal and/or mollusc-repellant agent, wherein the isolated compound is a terpene or oxygenated derivative thereof, the terpene is selected from one of the following general types:

- I) acyclic monoterpenes of the ocimene type;
- II) monocyclic sesquiterpenes of the bisabolene, germacrene or elemene type;
- III) acyclic sesquiterpenes of the farnesene type;
- IV) bicyclic sesquiterpenes of the santalene or carophyllene type; and
- V) furanosequiterpenes of the furanoelemene type, the furanoeudesma or furanogermacrene type.

- 26. The use according to claim 25 wherein the compound is combined with one or more carriers.
- 27. The use according to claim 25 or 26 wherein the compound is used in combination with one or more effector agents.
- 28. The use according to any of claims 24 to 27 wherein the molluscicidal and/or mollusc-repellant is effective against both aquatic and terrestrial molluscs.
- 29. The use of plant material derived from a plant in the plant family *Caesalpiniaceae*, *Olaceae*, *Polygonaceae* or *Bursecaceae* as an anti-barnacle agent.
- 30. The use according to claim 29, wherein the plant material is obtained from a plant in the genera *Eurymorpha*, *Alouatta*, *Leontideus*, *Leontideus* or *Leontideus*.

32. The use according to claim 31, wherein the plant is *Commiphora molmol* or *Commiphora guidotti*.
33. The use according to any one of claims 29 to 32, wherein the plant material is in the form of particles or a powder.
34. The use according to any one of claims 29 to 33, wherein the plant material comprises substantially the whole plant.
35. The use according to any one of claims 29 to 33, wherein the plant material comprises bark, leaves or a shoot of the plant.
36. The use according to any one of claims 29 to 32, wherein the plant material is an alcoholic extract of the plant.
37. The use according to claim 36, wherein the plant material is an alcoholic extract of the essential oil of the plant.
38. The use according to any one of claims 29 to 32, wherein the plant material is a substantially isolated compound.
39. The use according to claim 38, wherein the substantially isolated compound is used in combination with a carrier.
40. The use according to any of claims 29 to 39 wherein the plant material is used in combination with an effector agent.
41. The use according to any of claims 29 to 40 wherein the plant material is used as a paint-like preparation.
42. An anti-barnacle composition comprising an isolated compound and one or more carriers for use as an anti-barnacle agent, wherein the compound is a terpene or

oxygenated derivative thereof, the terpene is selected from one of the following general types:

- I) acyclic monoterpenes of the ocimene type;
- II) monocyclic sesquiterpenes of the bisabolene, germacrene or elemene type;
- III) acyclic sesquiterpenes of the farnesene type;
- IV) bicyclic sesquiterpenes of the santalene or carophyllene type; and
- V) furanosequiterpenes of the furanoelemene type, the furanoeudesma or furanogermacrene type.

43. The composition according to claim 42, which is suitable for applying to surfaces that come into contact with barnacles.

44. The composition according to claim 43 or 44, which contains between 0.1 and 50% v/v compound.

45. The composition according to claim 44, which contains between 3 and 25% v/v compound.

46. The composition according to claim 45, which contains between 6 and 25% v/v compound.

49. The use of an isolated compound as an anti-barnacle agent, wherein the compound is a terpene or oxygenated derivative thereof, the terpene is selected from one of the following general types:

- I) acyclic monoterpenes of the ocimene type;
- II) monocyclic sesquiterpenes of the bisabolene, germacrene or elemene type;
- III) acyclic sesquiterpenes of the farnesene type;
- IV) bicyclic sesquiterpenes of the santalene or carophyllene type; and
- V) furanosequiterpenes of the furanoelemene type, the furanoeudesma or furanogermacrene type.

50. The use according to claim 49, wherein the compound is combined with one or more carriers.

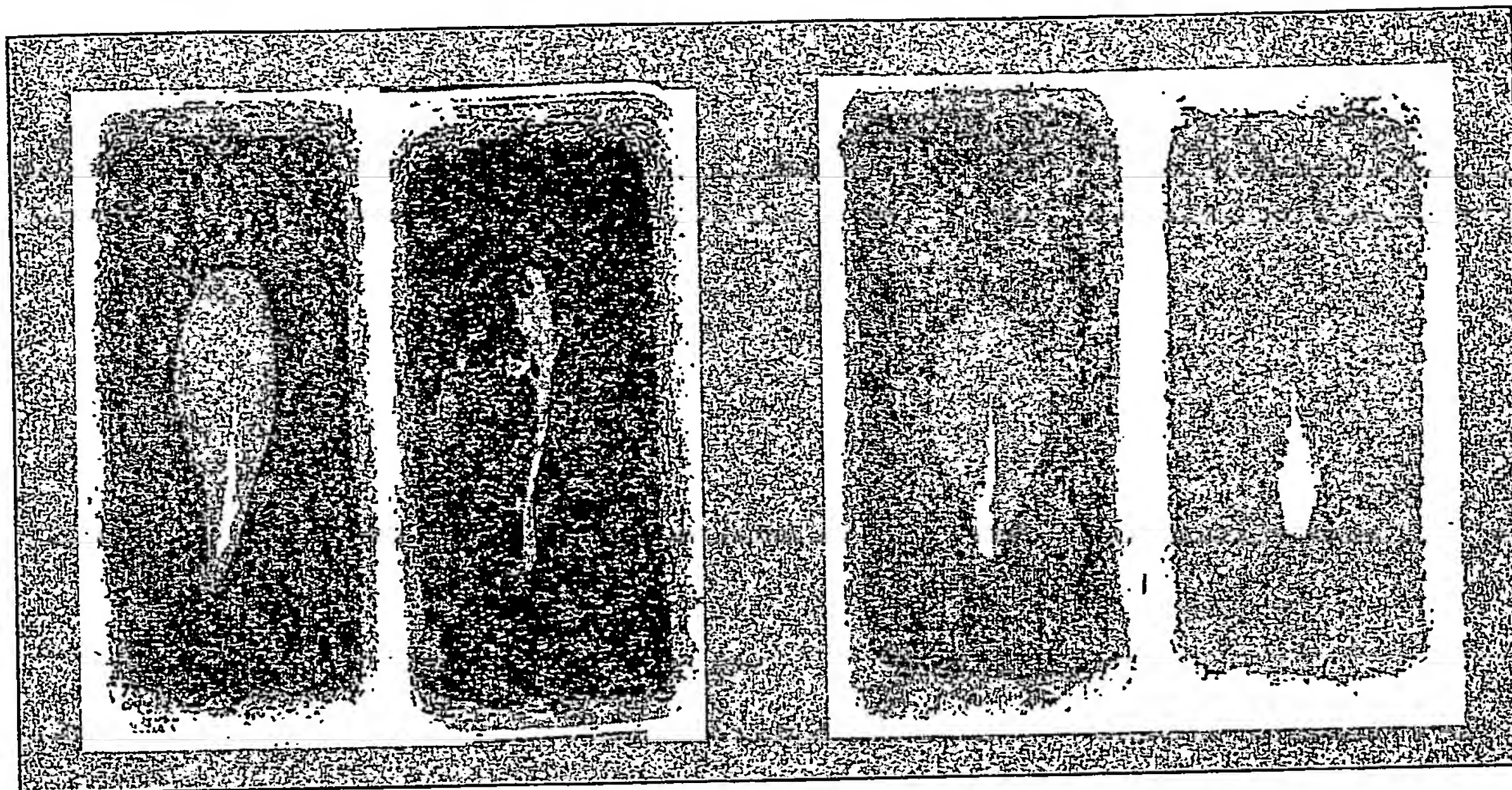
51. The use according to claim 49 or 50, wherein the compound is combined with an effector agent.

Figure 1

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Control (Water)
(Before) (After)

Aq. Ocimene (3%)
(Before) (After)



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Figure 2

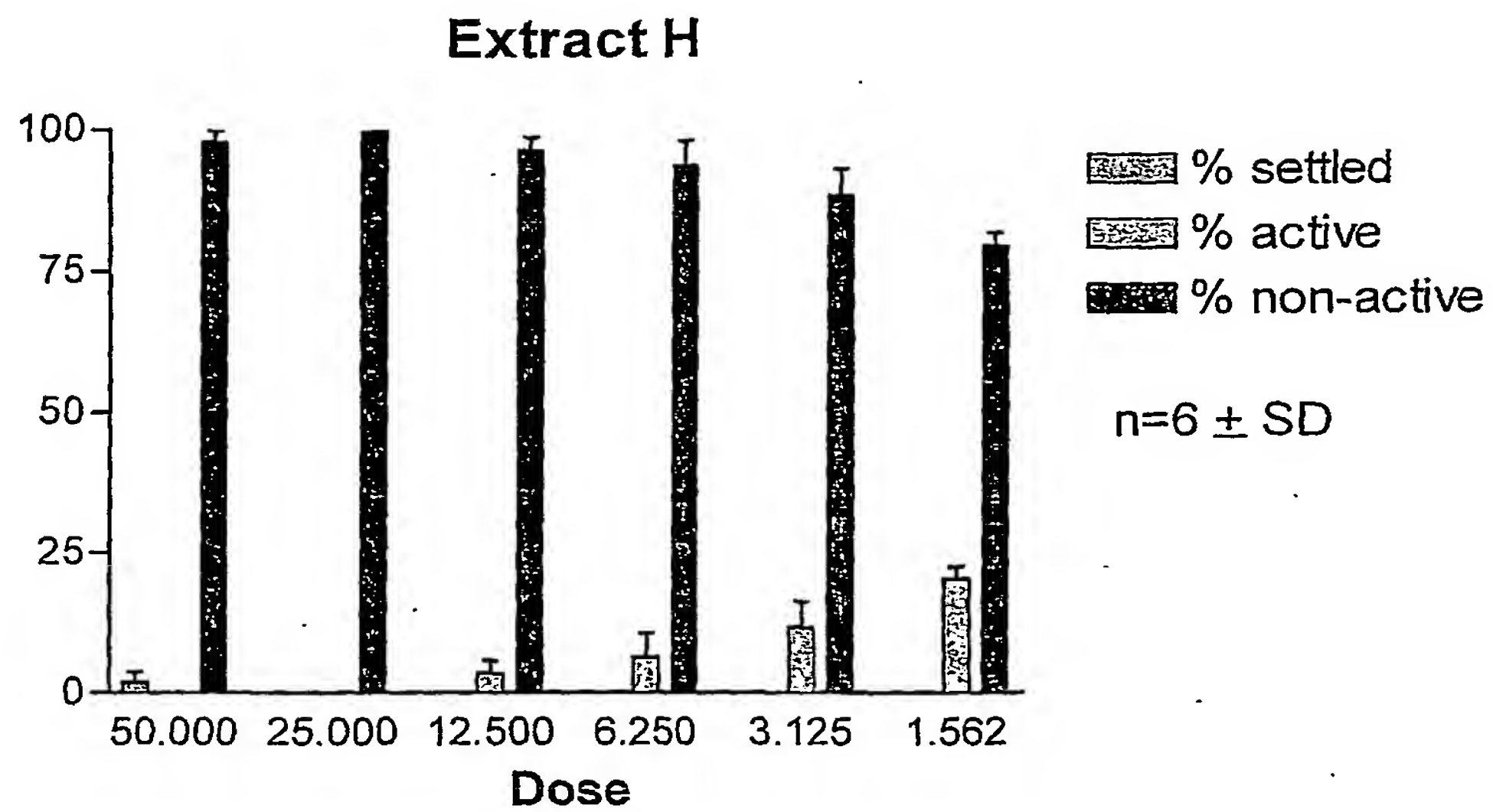
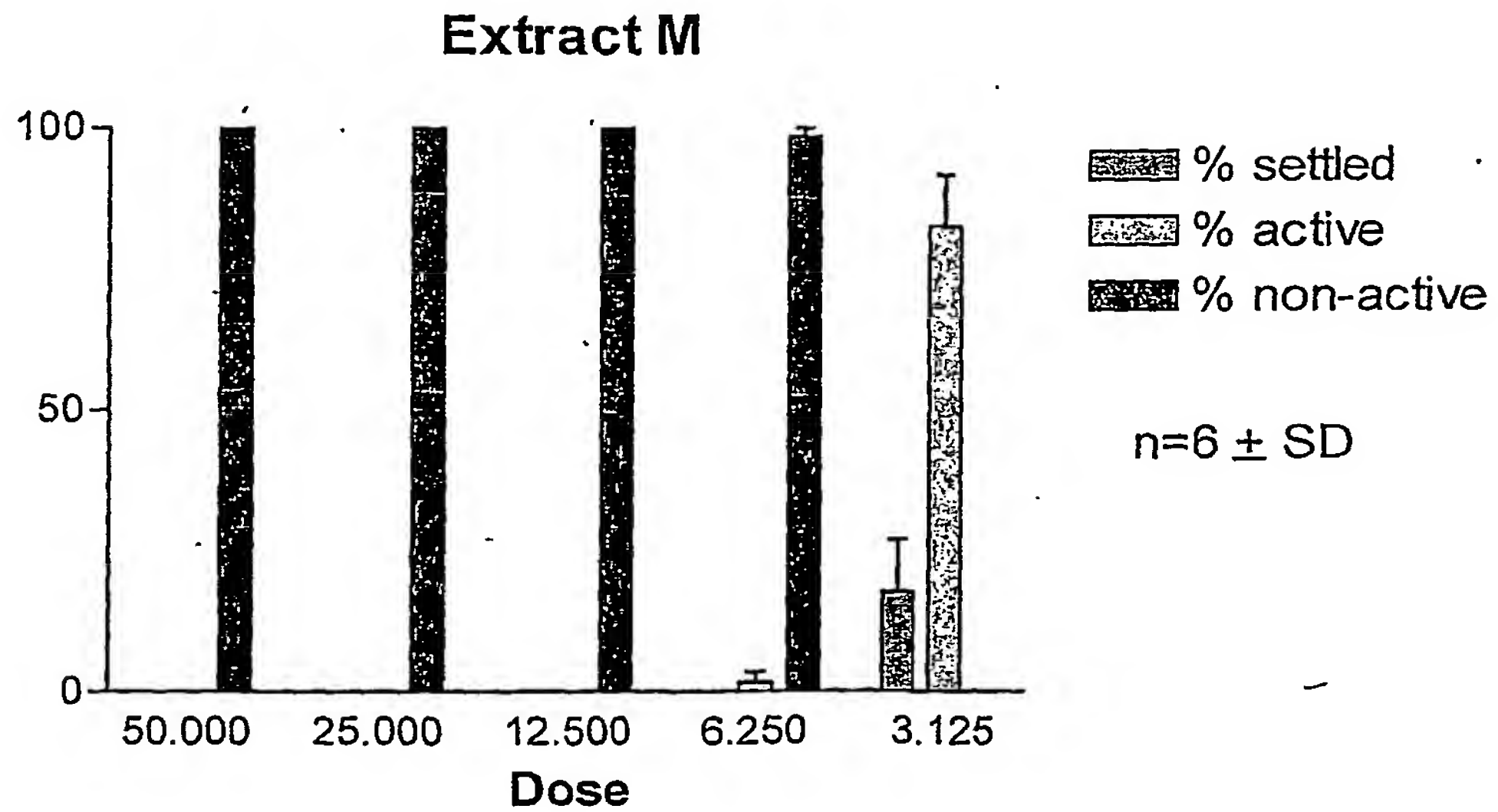


Figure 3



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Figure 4

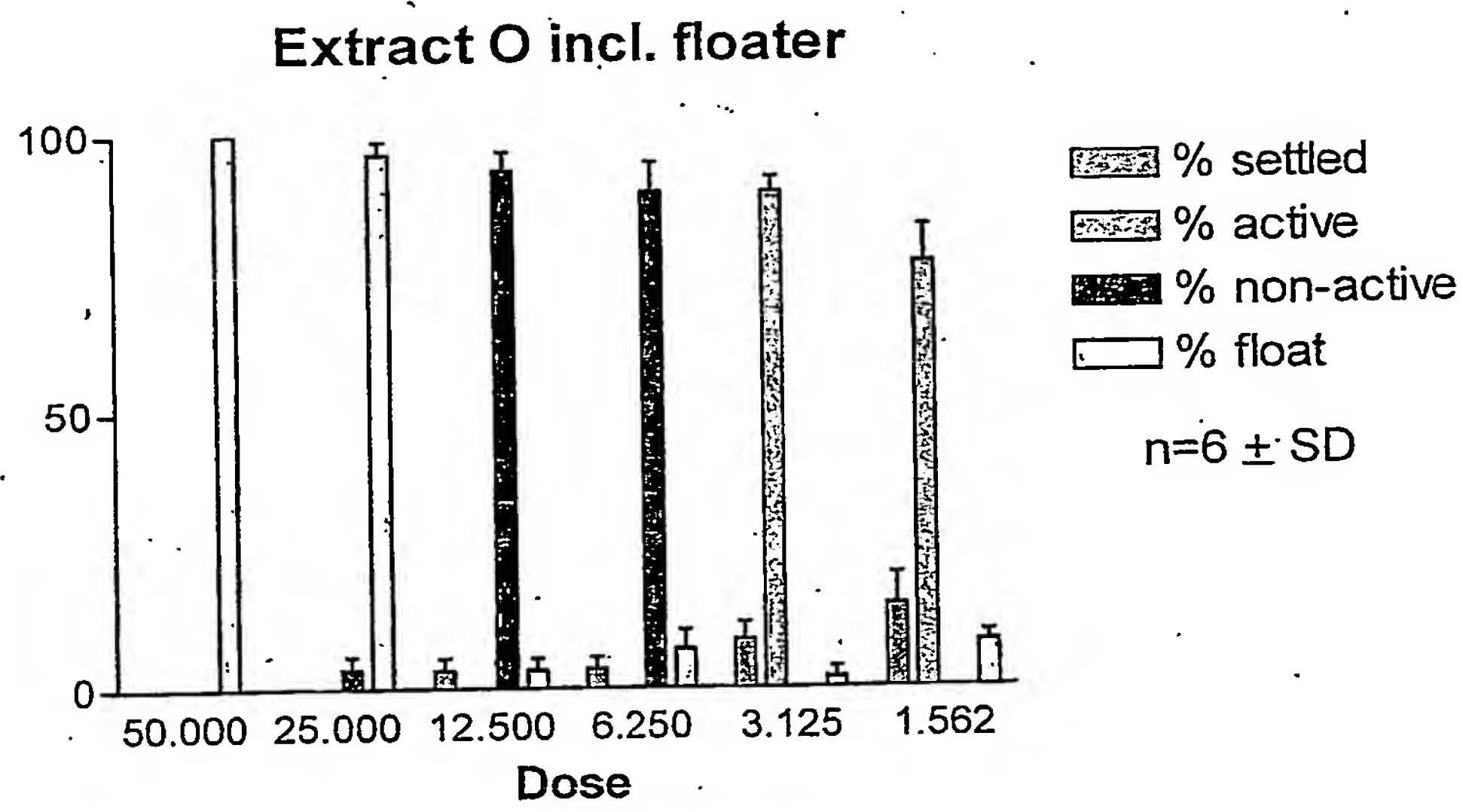
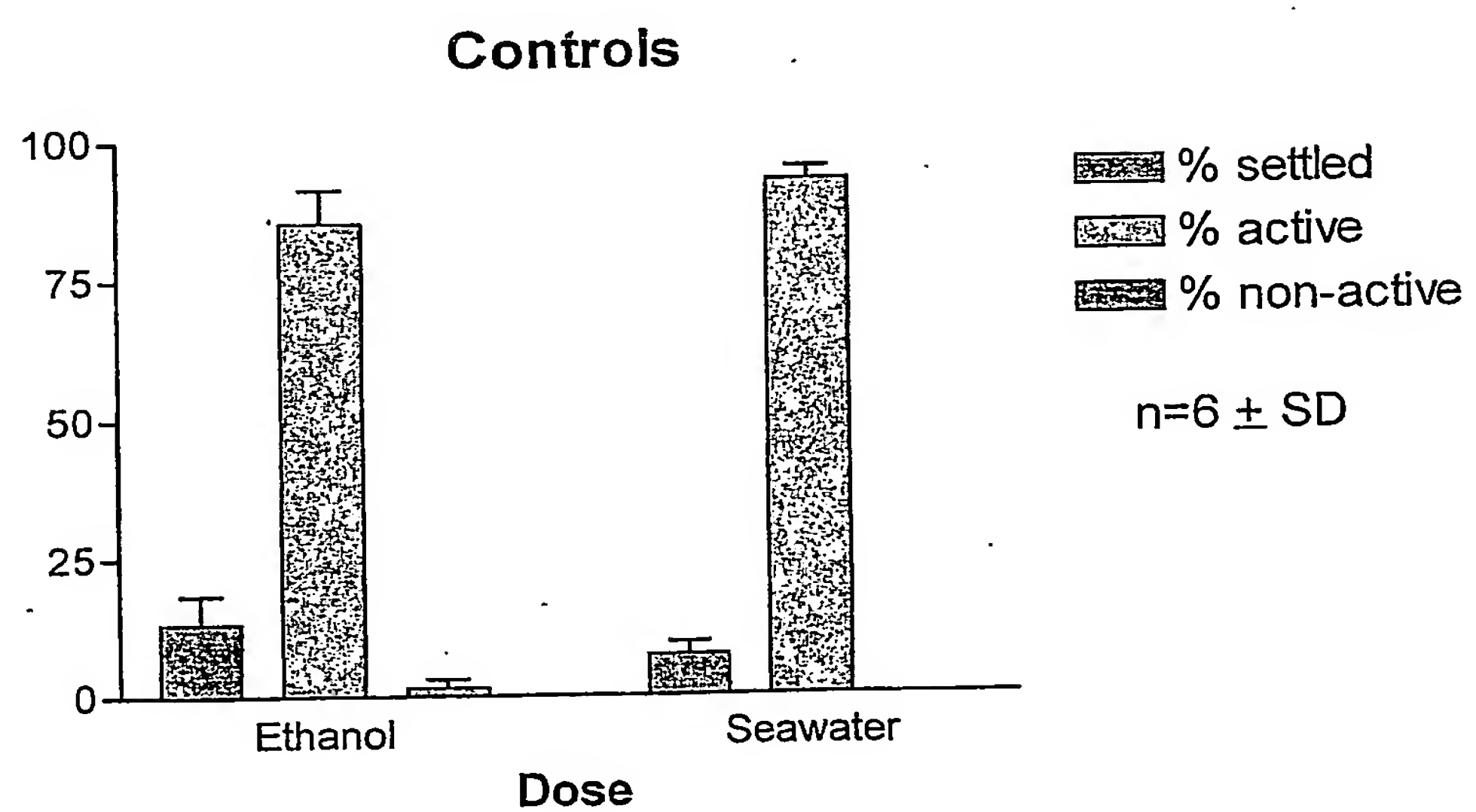


Figure 5



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